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IMMUNOHISTOCHEMICAL LOCALIZATION OF DIPEPTIDYL PEPTIDASE IV IN RAT DIGESTIVE ORGANS

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Immunohistochemical localization of dipeptidyl peptidase (DPP) IV in rat digestive organs was studied using the peroxidase-antiperoxidase (PAP method.

In the rat digestive organ observed, a fairly intense immunoreaction of DPP IV was found on the brush border of enterocytes of the small intestine. However, the immunostaining of enterocytes in the small intestine showed marked regional differences. The intensity of the reaction was significantly higher in the jejunum and ileum than in the duodenum. DPP IV was also detected in the cytoplasm of Paneth cells in the jejunum and ileum and on the surface of the crypt epithelium of the colon. In the esophagus, a faint reaction was detected on the surface of the stratified epithelium and connective tissues of the submucosa. DPP IV could not be found in the cells of the stomach by immunohistochemical means.

The present results suggest that DPP IV in digestive organs could mainly participate in the final digestion of peptides in which a proline residue is involved.

Dipeptidyl peptidase (DPP) IV (EC 3. 4. 14. 5) is one of a family of dipeptidyl peptidases that are characterized by their capacity to cleave dipeptide from an unsubstituted NH_2-terminus, and its action is specific for proline adjacent to the NH_2-terminal residue of the peptide (11, 15). The activity of DPP IV is widely distributed in various organs of vertebrates (4, 11). However, little is known about the physiological function of this enzyme.

In order to throw more light on the possible physiological function of DPP IV, we have studied the localization of this enzyme in various rat organs by an immunohistochemical method using an antiserum against highly purified DPP IV from rat kidney (1, 19).

The present paper describes the immunohistochemical localization of DPP IV and its activity in rat digestive organs.

MATERIALS AND METHODS

Materials: Five adult male rats (200–250 g) were used in the present study. Animals were fasted for 24 hr and killed by bleeding. The esophagus, stomach,

duodenum, jejunum, ileum, colon and pancreas were dissected out immediately. A portion of the specimens were used for the measurement of enzyme activity and another part were quick frozen in dry ice-acetone for light microscopic immunohistochemistry. In addition, for electron microscopic immunohistochemistry, small pieces of jejunum and ileum were fixed in periodate-lysine-paraformaldehyde (PLP) as described by McLean and Nakane (12) for 10 hr at 4°C.

Measurement of enzyme activity: The enzyme activity was assayed by the photometric method of Nagatsu *et al.* (13), using Gly-Pro-*p*-nitroanilide tosylate as the substrate. Protein was measured by the method of Lowry *et al.* (9) using bovine serum albumin as standard.

Light microscopic immunohistochemistry: For immunohistochemical observation, each tissue was cut into 6 μm-thick sections in a cryostat, which were transferred to non-precooled slides. After air drying for 30 min, the sections were fixed in cold chloroform-acetone (1 : 1) at 4°C for 5 min. To localize DPP IV, the peroxidase-antiperoxidase (PLP) method of Sternberger (22) was used. The details of the immunohistochemical procedures were described elsewhere (18).

Electron microscopic immunohistochemistry: After PLP fixation, the tissues were washed in 0.01 M phosphate-buffered saline (PBS), pH 7.4, containing 10% sucrose at 4°C for 12 hr and further incubated in a mixture of 20% sucrose and 10% glycerol in PBS for 12 hr. Then the tissues were embedded in O. C. T. compound and quick frozen. Eight-micron thick frozen sections were cut in a cryostat, transferred to albumin-coated glass slides, and air-dried at room temperature. They were then incubated in 10% hydrogen peroxide in methanol for 30 min at room temperature. After a 30 min incubation in 3% normal goat serum in PBS, the sections were incubated in a rabbit anti-DPP IV antiserum (1 : 2000) for 48 hr at 4°C. Subsequently, the sections were incubated at room temperature for 1 hr in a goat anti-rabbit Ig G serum (1 : 50) and then for another hour in a peroxidase-antiperoxidase (PAP) complex (1 : 100). After washing with PBS, the sections were postfixed in 2.5% glutaraldehyde in PBS for 1 hr. at room temperature. They were reacted with 3,3′-diaminobenzidine (DAB) containing 0.005% H_2O_2 for 20 min at room temperature. Then the sections were washed, osmification in 1% osmium tetroxide in PBS at room temperature for 1 hr dehydrated in a graded series of alcohols, and embedded in TABB's embedding resin. Ultrathin sections were examined with a JEM 100 B electron microscope without additional staining.

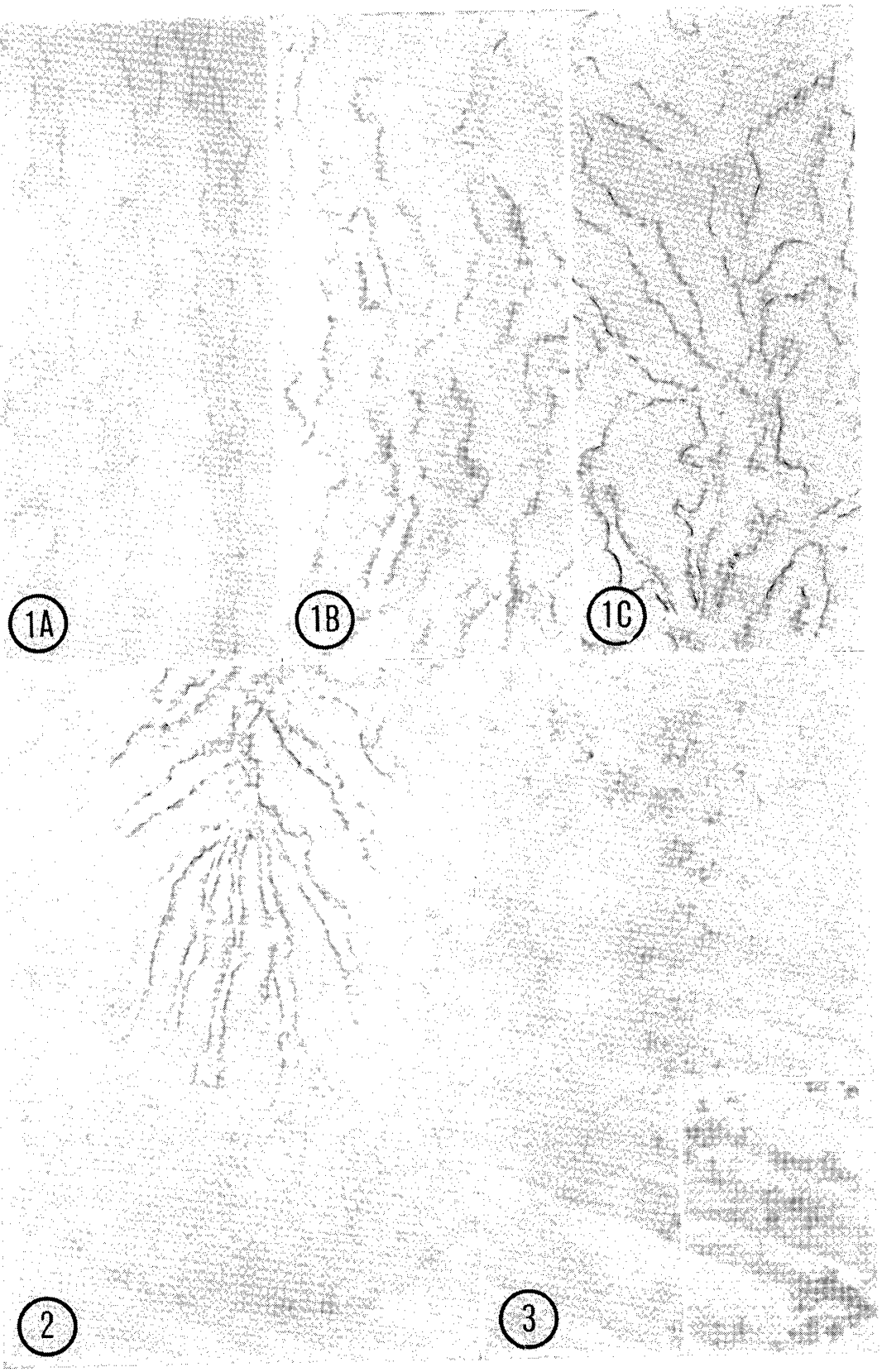
Control experiments: Control experiments, both for light and electron microscopic immunohistochemistry, were performed by substitution of the rabbit antiserum against DPP IV with normal rabbit serum and with an antiserum preabsorbed with purified DPP IV.

Antisera: The antiserum to the highly purified DPP IV from rat kidney was raised in a rabbit, and its specificity has been described previously (1). Goat anti-rabbit Ig G and the peroxidase-antiperoxidase (PAP) complex preparation were obtained commercially from Miles Laboratories.

RESULTS

Immunohistochemical observations

In the rat digestive organs observed, a fairly intense immunoreaction of DPP IV



was found on the brush border of enterocytes of the small intestine. However, immunostaining in these cells showed marked regional differences. As shown in Fig. 1A–C, the intensity of the reaction was significantly higher in the jejunum and ileum compared with that in the duodenum. Furthermore, the present study revealed local variation of the enzyme in the intestinal villi in that the immunoreaction of DPP IV was most intense in the upper part of the villi, and no or minimal reaction was found in the epithelial cells of the crypts (Fig. 2). DPP IV was also detected in the cytoplasm of Paneth cells in the jejunum and ileum (Fig. 3). In the esophagus, a faint immunoreaction was detected on the surface of the stratified epithelium and connective tissues of the submucosa (Fig. 4), while DPP IV could not be found in the cells of the stomach by immunohistochemical means (Fig. 5). A fair degree of DPP IV immunostaining was present on the crypt epithelium of the colon (Fig. 6). In the pancreas, localization was restricted to the luminal surface of interlobular and intercalated ducts, and a faint reaction was also found in the capillary endothelium in the islet of Langerhans as described previously (19) (Fig. 7).

By electron microscopic immunohistochemistry, DPP IV was identified on the external surface of brush border membranes of enterocytes in the jejunum and ileum (Fig. 8). No immunoreaction could be found in the cytoplasm of these cells by the present immunocytochemical method.

In control sections treated with normal rabbit serum or with a preabsorbed antiserum the reaction was negative in all organs tested.

Biochemical Study

DPP IV activity (μm mol/min/mg protein) in homogenates of various rat digestive organs was also measured biochemically. These results generally agreed well with those of immunohistochemical observations. In the digestive organs presently measured, the highest activity was found in the small intestine. Particularly, the activity of DPP IV in the jejunum (29.4) and ileum (32.1) were significantly higher than that in the duodenum (17.0).

DISCUSSION

The present immunohistochemical study demonstrated that in the rat digestive organs DPP IV was mainly localized on the brush border of the small intestine, while an intense immunostaining was also found on the luminal membrane of intercalated and interlobular ducts in the pancreas. These results generally agree well with those reported recently by Gossrau (3), who used a histochemical technique. However, there were some discrepancies in its localization between the two studies. Marked differences in the localization of DPP IV were chiefly found in the small intestine: 1) Gossrau reported that DPP IV activity was found in the Golgi region

FIG. 1. Intestinal villi of the duodenum (A), jejunum (B) and ileum (C). DPP IV is localized on the brush border of the enterocytes. The intensity of the immunoreaction is significantly higher in the jejunum and ileum than the duodenum. ×120

FIG. 2. Ileum. Fairly intense immunoreaction of DPP IV is found on the brush border in the villi, and no or minimal reaction is detected in the crypt epithelial cells. ×60

FIG. 3. Ileum. Immunoreaction of DPP IV is seen in the cytoplasm of Paneth cells. ×250 Inset, higher magnification. ×600

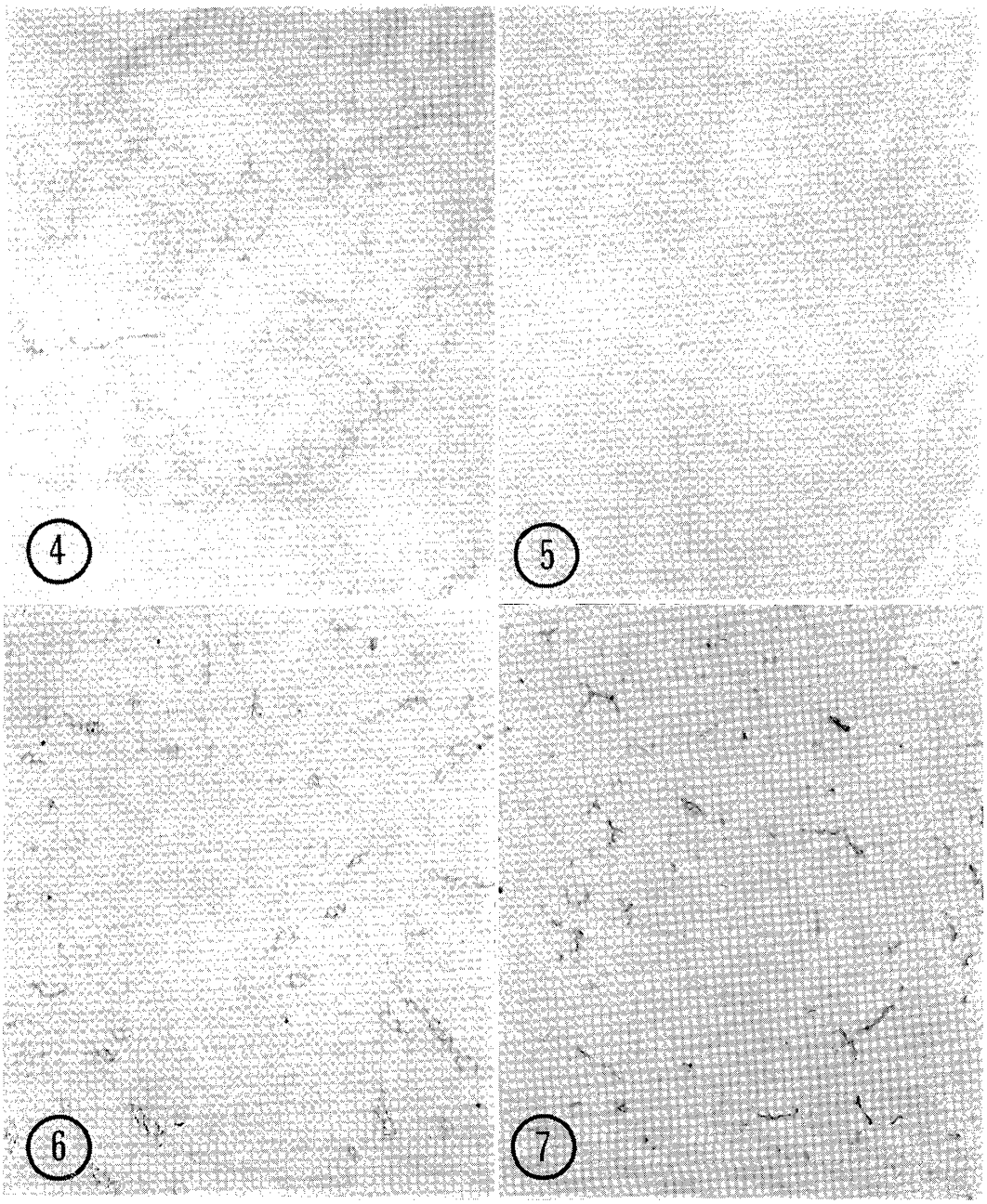


FIG. 4. Esophagus. Weak stainings are found on the surface of the stratified epithelium and connective tissues of submucosa. ×50

FIG. 5. Stomach. DPP IV can not be detected in any cell by immunohistochemical means. ×80

FIG. 6. Colon. Fair immunoreaction of DPP IV is found on the surface of the crypt epithelium. ×60

FIG. 7. Pancreas. DPP IV is restricted to the luminal surface of intercalated and interlobular ducts. Weak reaction is also seen in the capillary endothelium. ×100

of the enterocytes in the lower and middle third of the jejunal and ileal villi. However, in our immunohistochemical studies, both at the light and electron microscopic level, DPP IV could not be detected in the Golgi region of any enterocytes.

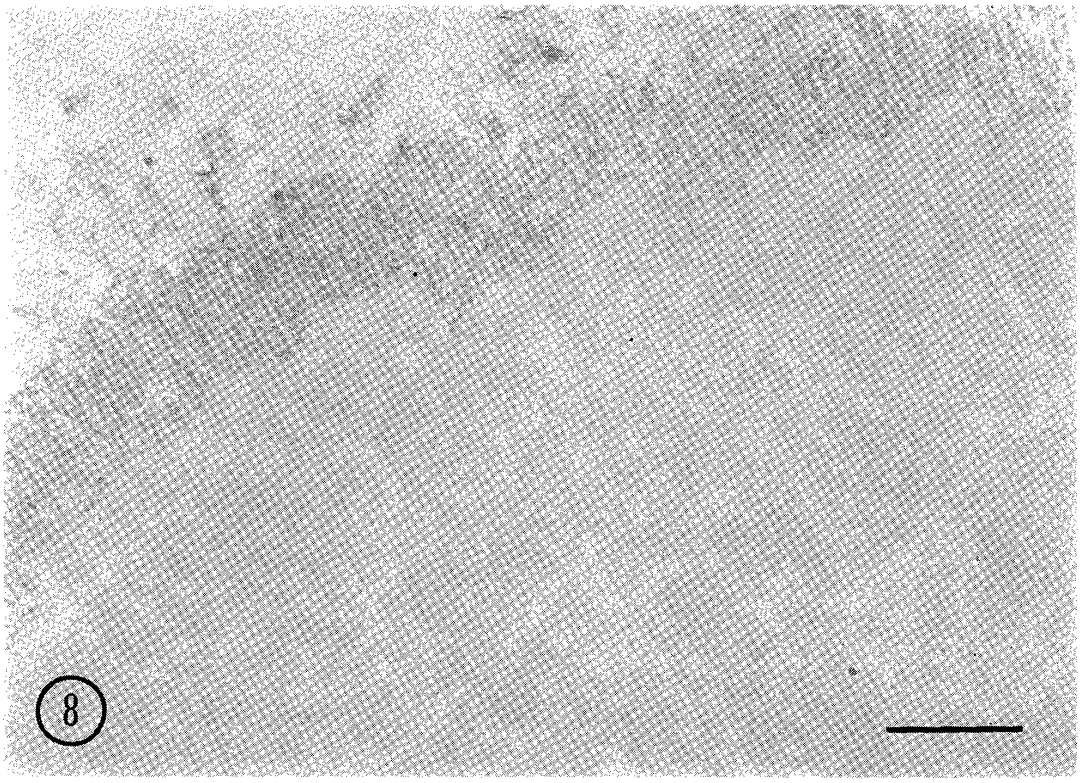


FIG. 8. Immunoelectron micrograph of the enterocytes of the ileum. Immunoreaction is restricted to the external surface of brush border membrane. However, no reaction can be seen in the cytoplasm of these cells. Bar=1 μm

2) In the histochemical study intraepithelial and stromal lymphocytes showed DPP IV activity on their surface membranes, while we could not find any immunostaining in these cells by immunohistochemical means. 3) The results of the present study demonstrate local variation of the enzyme in the intestinal villi in that the immunoreaction of DPP IV was most intense in the upper part of the villi, and no or minimal reaction was found in the epithelial cells of the crypts. On the other hand, Gossrau reported DPP IV in the lower third of the intestinal villi and in epithelial cells comprising the crypts. These discrepancies might be due to differences in the staining methods used, since both studies used the same fixation procedures.

In general, immunohistochemistry is based on the antigenic nature of the enzyme and can not give primary information about enzyme activity. However, it is not reasonable that DPP IV activity should be found in the Golgi region of enterocytes and on the surface membrane of lymphocytes in the small intestine by using a histochemical method, while these sites were completely free of immunoreaction in the present study. If DPP IV activity was really localized in these sites, immunostaining for the enzyme should be found in the same sites. Therefore, two possible reasons for this discrepancy should be considered. The reaction products of DPP IV in the Golgi region of the enterocytes and on the surface membranes of lymphocytes in the small intestine might be due to an isoenzyme or are artifacts due to other peptidases.

It is well-known that DPP IV is found in kidney and intestinal brush border

along with some peptidases such as peptidase A and peptidase M (5). Svenson *et al.* (23) purified DPP IV from pig small intestinal brush border membrane. DPP IV activity has also been localized *in situ* on the brush border of enterocytes of the small intestines of some animals, including humans, by the use of histochemical methods (2, 3, 8). In the present study, it was revealed that DPP IV in the rat small intestine was restricted to the external surface of brush border membranes in the enterocytes, thus confirming previous studies. Furthermore, it was demonstrated that DPP IV in the small intestine showed marked regional differences. The intensity of the immunoreaction, as well as DPP IV activity, in the jejunum and ileum was significantly higher than in the duodenum. These results suggest that DPP IV in the small intestine might work on the degradation of proline-containing peptides derived from the final products of intraluminal protein digestion by gastric and pancreatic proteinases. Only very specific enzymes are able to split a bond in which a proline residue is involved (6, 21), and gastric and pancreatic proteinases are not capable of splitting such peptide bonds.

It has been suggested that di- and tripeptides can be transported into the intestinal cells by specific carrier systems which are different from those involved in the transport of free amino acids (10). It is noteworthy that glycyl-proline is a dipeptide very poorly hydrolyzed by brush border enzymes (16) and has affinity for the dipeptide carrier system of the small intestine (7, 17). DPP IV cleaves NH_2-ternimal dipeptides, X-Pro, from polypeptides, and Gly-Pro-Y is known to be one of the most suitable substrates for this enzyme (11, 15). Furthermore, it has been suggested that glycyl-proline hydrolysis is accomplished entirely intracellularly (14, 20). These lines of evidence suggest the possibility that DPP IV on the brush border membrane of enterocytes in the small intestine may not only work on the digestion of proline-containing peptides, but may also play a significant role in the dipeptide carrier system.

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